MAGNETIC CONTROL OF MICRO-MAGNETIC BEADS WITH ATTACHED ENZYMES AND CATALYSTS

By

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Submitted to the Department of Biological and Environmental Engineering in partial fulfillment of the requirements for the Degree of

Master of Engineering

In Biological and Environmental Engineering

Cornell University

January 2014

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Biographical Sketch

Vishal Bhagwandas was born in Queens, NY on February 25, 1989 to Ravi and Kamla Bhagwandas. Vishal has always shown to be curious and inquisitive at a young age. He showed a strong affinity for math and science since elementary school. In high school, Vishal excelled at math and science both inside and outside the classroom. His interest in helping others and wanting to make a lasting impact led him to pursue higher education in the sciences. In the fall of 2007, Vishal entered Cornell as freshman with the intention on focusing his studies on Biological Engineering. Four years later in May 2011, he graduated with a Bachelors of Science. After a year of volunteer work and bioinformatics research, Vishal decided to pursue a Masters of Engineering Degree at Cornell University in Biological Engineering to gain further skills and knowledge with application.

Acknowledgements

I would like to acknowledge Daniel Aneshansley for his advice, guidance and most of all his patience with me throughout my entire experience in the graduate program. I would also like to say how grateful I am for taking me on as his advisee. I would like to thank Stephanie Corgie for allowing me to use his work for this project. Finally, I would like to give thanks to my family and friends who have always given me support and advice. A special thanks goes to my girlfriend Emily who has always provided me with much support, guidance, encouragement and assistance.

Table of Contents

List of Tables

List of Figures

ABSTRACT

The development of magnetic beads in recent years has allowed for manipulation and transport of bio molecules which are attached to the magnetic beads. By attaching enzymes to the magnetic micro beads, they can be used in solution as a bioreactor, with the mixing and agitation controlled by electromagnets. A method was developed using video footage and ImageJ to capture and analyze the movement of micro magnetic beads in solution. The method was used to analyze the motion of micro magnetic beads in solution developing steady state mixing. Once steady state mixing has been achieved, this method was used to examine the properties of micro magnetic beads in solution by controlling the strength of the electromagnets, frequency of mixing, and concentration of the micro magnetic bead solution. Preliminary results suggest favorable mixing with stronger magnetic fields and higher frequency of agitation.

II. Introduction

II.1 Magnetism

The phenomena of magnetism includes forces or repulsion and attraction that are exerted by magnets on other magnets and paramagnets. A magnet is a material or object that produces a magnetic field. The magnetic field is responsible for the force that pulls, attracts and repels other magnets or ferromagnetic materials such as iron.[1]

Several types of magnets exist in the world. Permanent magnets creates their own persistent magnetic field. Ferromagnetic materials are highly susceptible to become magnetized with soft materials tending to be weakly magnetized and hard materials holding the magnetism well.[1] And finally, the electromagnet which is made from a coil of wire that act as a magnets only when current passes through the wire. Electromagnets are often wrapped around soft ferromagnetic materials. By doing this, it can greatly enhance the magnetic field that is produced from the current in the coil.

The strength of a magnet is measured by the magnetic field that it produces. The strength of the magnetic field is measured in teslas [T]. Magnetic Flux is the measure of the flow or lines of force; this is also known as the total amount of magnetism. The unit of measure of magnetic flux is weber [Φ]. Magnetic flux density is simply the total flux divided by the cross sectional area which is given in teslas.

II.2 Magnetic Beads

The development of magnetic beads in recent years has allowed for manipulation and transport of bio-molecules which are attached to the magnetic beads. They are typically of 0.5 to 500 micrometers in diameter. Due to the diameter of these microbeads and how they are made; they typically have very large surface area to volume ratios which provides large surface area for attachement of bio-reactive materials in much smaller volume.[2]

For a sphere, the surface area to volume ratio is 3/R where R is the radius of the bead. As R becomes smaller the surface area to volume ratio increases such that with bead of 5.0 microns in diameter has a ratio which is 10^3 greater than sphere of 5.0 millimeter diameter. To fill the volume of a sphere 5.0 millimeters in diameter would require $740*10^6$ beads of 5 micron diameter with an increased surface area equal to $740*10^9$ times that of the 5 millimeter diameter bead.

They have other properties that make them very useful in biological applications. They are small superparamagnetic microparticles which means that it can flip magnetic direction under the influence of temperature.[3] Due to their size, they are highly susceptible to magnetic fields and are able to maintain their magnetic domain under excitation. As the magnetic field is removed, the magnetic beads loses all magnetization and has no residual force left.[3] After the magnetic field has been removed, the magnetic field will redisperse in the suspension of liquid.

Those properties allow for some useful applications within biomolecular biology and bioengineering. Magnetic beads can be coupled with bio-reactive materials such as an antibody, streptavidin, protein, antigen, DNA/RNA, and forms of enzymes.[2] By doing so one can use Magnetic Beads for bioseparation. By attaching a antibodies to a magnetic beads, one can separate out specific biological molecules with the use of stronger magnets. One can also use magnetic beads to wash any materials that is bound to it by quickly applying and removing magnetic fields with different polarity. Magnet beads coupled with streptavidin is a very efficient way to isolated biotinylated molecules. When used with DNA/RNA protein binding studies, it can be very useful for sequencing, and preparing single stranded templates.[2] A very useful way to use magnetic beads would be to attach an enzyme to them for use in a bioreactor.

Randomly distributed magnetic microparticles (MNPs), which are simply nano-sized magnetic beads can have a major role in chemical and biochemical reactions.[1] There has been reported activity in large magnetite nanoparticles that did not contain any enzyme and followed Michaelis-Menten kinetics with peroxidase like activity. In particular, the Horseradish Peroxidase enzyme has been observed to work well with MNPs.[1] This enzyme is a part of a class of enzymes that reduces H_2O_2 to H_2O so that it can aromatize aromatic compounds. Horseradish peroxidase has been widely studied because of its relevance and applications in biomedical engineering, environmental technologies and industrial biotechnologies. In a paper by Corgie et al., they examine the use of MNPs, embedded with Horseradish peroxidase in order to improve its kinetic model or simply to increase the efficiency that this enzyme operates with the substrate.

In this study, the authors formed a Bio-nanocatalysts (BNCs) with horseradish peroxidase embedded with MNPs. Enzyme activity, inhibition and turnover were tested in this study with different sizes of embedded BNCs. Experiments were conducted again with both horseradish peroxidase and MNPs and compared with the BNCs. With the BNCs it showed more activity than the enzyme alone and there is no loss of catalytic activity and can be easily recovered and reused as catalyst.[1] As a result, bioreactions involving this enzyme can be done much more efficiently with faster reactions, lowered inhibitions and minimal loss of activity and be easily recycled over and over again. They found that BNCs had enhanced enzymatic activity and overall much more active than the free enzyme or the MNPs. The use of enzyme embedded magnetic beads can allow for the creation of magnetic bioreactors that could operate much faster and efficiently than traditional bioreactors used today in industrial applications.

II.3 Technologies for Producing Magnets and Electromagnets

Permanent magnets are made with hard crystalline iron ferrite mineral, also known as magnetite. Ceramic permanent magnets are made from finely powered particles with a strong magnetic field during formation. Recent developments allowed permanent magnets to be made with powdered samarium cobalt fused under heat. This aligned the magnetic domains to be aligned naturally and thus very strong magnets to be created that were much smaller.[4]

The manufacturing process is relatively straightforward for permanent magnets. The first step consists of melting the materials in a vacuum. Once cooled and solidified, the materials are broken up and crushed into powdered metal. After that, the powdered metal is placed in a mold and a magnetic field is applied to align the particles magnetic domains and then it is pressed with pressures between 10,000 and 15,000 psi. The pressed material is then heated to create a solid piece of metal for the magnet.[4] A second heating and cooling session is done to remove residual stress and strengthens it. The magnet is then finished into the desired shape. Afterwards, the magnet is magnetized between two strong magnets for a period of time,[4] so that atoms can align thus truly making the piece of metal into a magnet.[5]

Electromagnets are made by wrapping soft ferromagnetic material with coils of wire. This phenomen works since current passing through a wire produces a magnetic field. By wrapping several coils (solenoids) this further increases the strength of the magnetic field that is produced.[6] The soft ferromagnetic material that the solenoid covers can produce a great magnetic field once it is first applied to the magnetic. So by running current through the solenoid that wrap around the soft ferromagnetic material, it activates that magnet and thus creating a greater magnetic field that the coils can produce. The higher number of coils of the solenoid, the greater the strength of the electromagnet. By replacing the solenoids with Bitter plates[6] one can create much stronger electromagnets that can withstand the pressure and heat generated from the magnetic fields.[6]

The technology to produce micro magnetic beads was developed by Dr. John Ugelstad in 1976 shortly after he developed the technology to create uniform polystyrene beads of the same size.[7]

III. Objectives

This project will examine the magnetic control of Micro-Magnetic Beads with attached enzymes and catalysts. It will analyze the effects of different using electromagnets to control the micro magnetic beads in this project. The specific objectives are to:

- Develop a method for imaging and analyzing the movement of micro magnetic beads in cuvette.
- Examine the properties of micro magnetic beads in solution such as
    - Magnetic field strength by varying the voltage across the electromagnet.
    - Frequency at which the voltage is switched on and off to the electromagnets.
    - Impact of different concentrations of micro magnetic beads.

IV. Materials and Methods

IV.1 Dynamic Magnetic Trap Bioreactor System

The DMTB system was developed by Daniel Aneshansley and Stephane Corgie. The system consisted of electromagnets, simple microcontroller (Arduino UNO - http://arduino.cc/en/Guide/HomePage) and interface circuits between the microcontroller and electromagnets. The electromagnets are mounted on a custom stand which aligns the electromagnets on each side of a disposable UV transparent plastic micro cuvettes with 0.5 cm cross-section, which is pictured below (Figure 1). The microcontroller can be programmed to control the frequency, amplitude and signal timing for controlling the electromagnets. The circuit used to control the electromagnets is below (Figure 2).

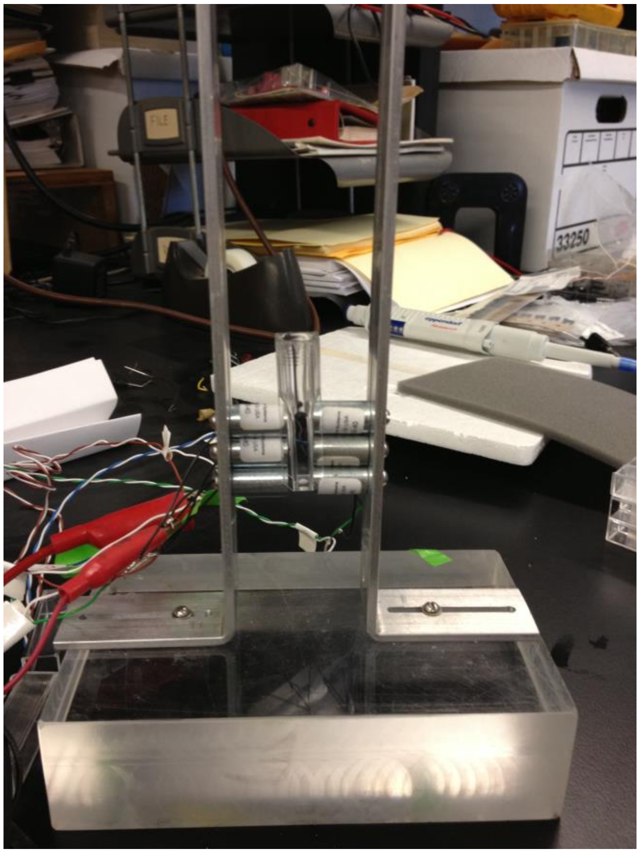

Figure 1. The Dynamic Magnetic Bead Bioreactor

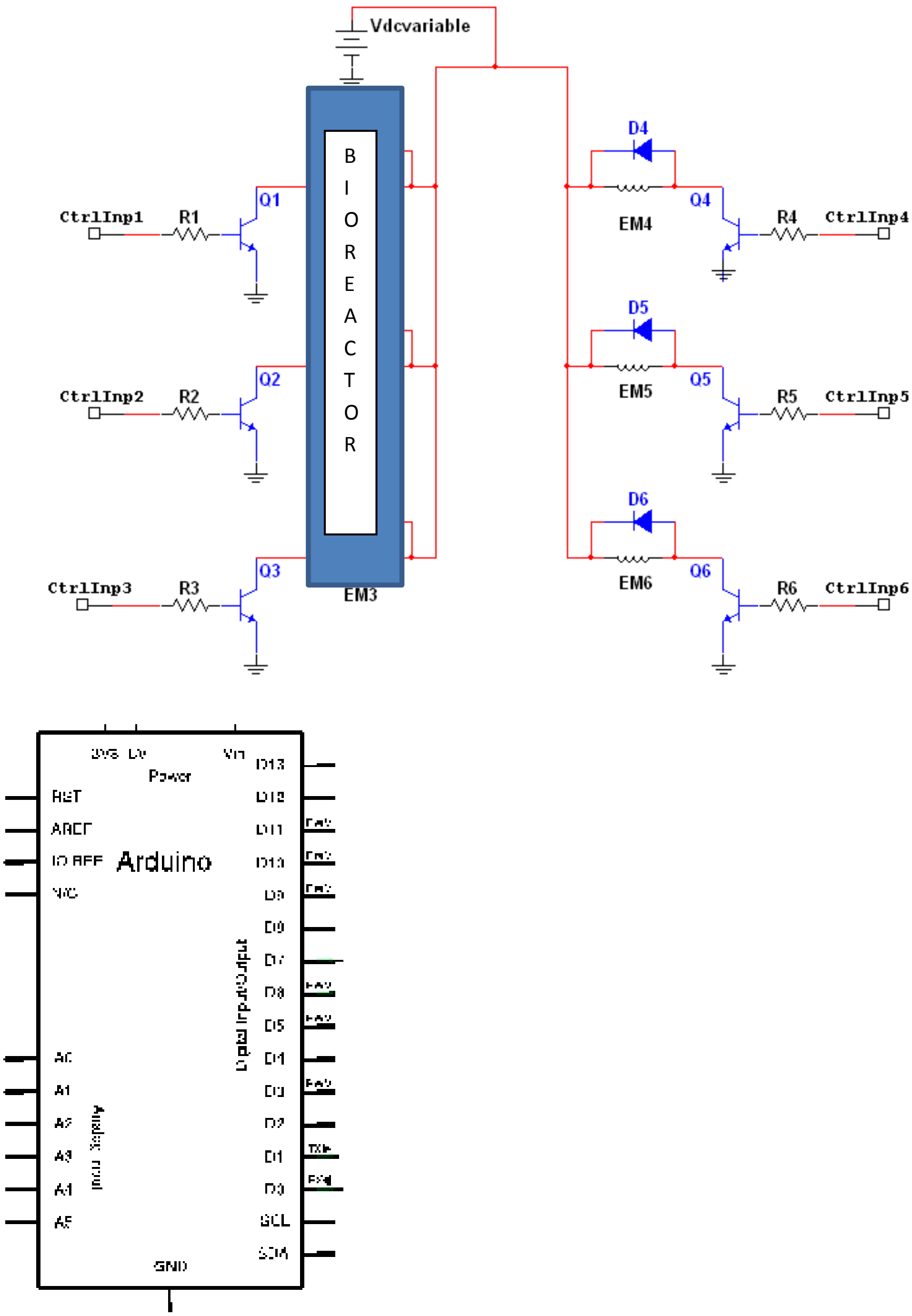

Figure 2. Schematics of the interface citcuits with bioreactor and Arduino Uno below. CtrlInp come from a microcontroller like the above Arduino.

IV.2 Micro Magnetic Beads, Cuvettes & Electromagnets

For this project, I used magnetic beads from Sigma-Aldrich with a product number of 310069. These are simply Iron (II,III) oxide (Fe_3O_4) that visually as a black powder. The average radius is ≤ 5 microns. They have a density of 4.8-5.1 g/mL at 25 °C. A bead with a 5 micron radius has a volume of ca 20×10^{-12} cm^3. The number of these beads in a cm^3 or mL is about 74% of this volume divided into 1 cm^3 because of air space between beads or about 37 billion ($.74*50*10^9$). Therefore each bead weighs about $0.135 * 10^{-9}$ grams.

The electromagnets were tubular 1 cm diameter rated for 12VDC at 50% duty cycle with a coil resistance of 127 ohms. (E-66-38-40 from http://www.electromechanicsonline.com) which could hold 15.6 oz or 4.3 N).

The cuvettes used in this project are 1.5mL microcuvette made from polystyrene(112137, Globe Scientific) is with a rectabgular shape and a 0.5 cross section perpendicular to the electromagnets magnetic axis.

IV.3 Micro Magnetic Beads Solutions

A solution of magnetic beads was created using 5 mL of distilled H_2O and 0.05 g of the micro magnetic beads; this gives a mass concentration of $\rho = 10$ g/L. A solution with a concentration of $\rho = 10$ g/L was alsol prepared in a simliar fashion. Using a micropipette, 1500 uL was drawn from the solution and dispenced it into the micro cuvette. As a pretest, the magnetic beads were able to all be controlled in the micro cuvette by placing a permanent magnet around it and moving it.

IV.4 Control of Switching Frequency and Magnetic Strength of Electromagnets

In order to study the properties of the micro magnetic beads in solution, a program was written for the Arduino Uno controller so that it would alternate electromagnetic pulses with the electromagnets at different frequencies (Appendix A). This was written so that while one electromagnet turned on, the electromagnet across from it would be off and vice versa. An oscilloscope (Tektronix DPO 2020) was used to measure the voltage that is sent to the electromagnets as the power supply voltage was adjusted. By increasing the voltage, sent to the electromagnet, this would increase the strength of the magnetic fields generated. The maximum voltage used with the electromagnet was 12 V as it was rated for that voltage at a 50% duty cycle.

IV.5 Experiments Performed

The solution was well mixed using the pipette and placed into the custom stand in between the electro magnets. The program of the microcontroller was written so that it would pulse at specified frequencies. Steady-state mixing is defined in this project as when the magnetic beads would move back and forth between the sides of the cuvette until no more beads would fall to the bottom of the cuvette. Video was captured with an iPhone camera with overhead lighting from the room and recorded the solution until it reached a steady state of mixing for the electromagnets pulsing at frequencies $f = 2.00$ Hz, 0.33 Hz and 0.17 Hz. This experiment was repeated for the electromagnets operating at these voltages: 12 V, 10 V, 8 V, and 6 V. This experiment was performed with solutions of a mass concentration $\rho = 5$ g/L and 10 g/L.

IV.6 Examining the Motion of Micro Magnetic Beads

Motion of the micro magnetic beads was examined as a function of the switching frequency and voltage of the electromagnet. A solution of micro magnetic beads at the mass concentration of $\rho =$ 10 g/L was used to examine changes in motion of micro magnetic beads with the electromagnetic pulsing at a frequency, f = 2.00 Hz and the voltage at which the electromagnets operated at were at 12 V, 10 V, 8 V, 6 V, 4 V, and 2 V. The cuvette was filled with 1500 uL of solution of magnetic beads and placed in between the electromagnets as pictured below (Figure 3) and after stead-state has been achieved, video was recorded the steady state mixing that occurred.

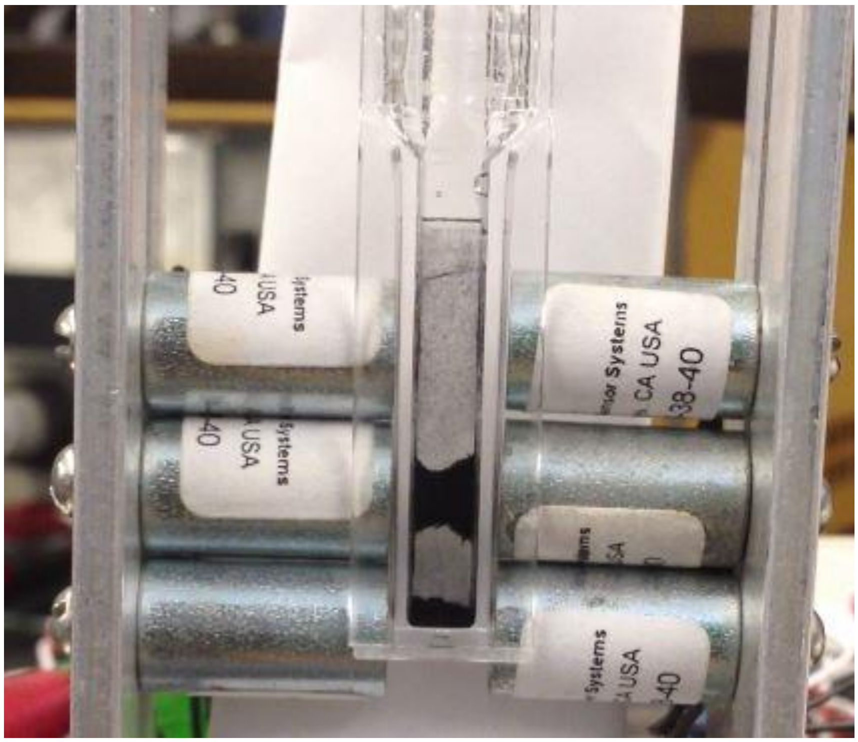

Figure 3. The micro cuvette with a 1.5mL solution of micro magnetic beads during steady-state mixing with the electromagnets operating at 12V and pulsing at a frequency of 0.5Hz.

This protocol was repeated for the electromagnets pulsing at frequencies, f = 1.00 Hz, 0.50 Hz, 0.33 Hz, 0.25 Hz, 0.20 Hz, and 0.17 Hz with 50% duty cycle. (Table 1 below of time between pulses & frequency)

Table 1. Seconds between the swtiching of the electromagnets and their corresponding frequency

Time Between Pulses [s]	Frequency [Hz]
0.5	2.00
1	1.00
2	0.50
3	0.33
4	0.25
5	0.20
6	0.17

The experiment was repeated for f = 1 Hz and at 12V, 10V, 8V, 6V, 4V, and 2V at micro magnetic bead concentration half of the original solution mass concentration, $\rho = 5$ g/L and double the initial mass concentrations, $\rho = 20$ g/L.

IV.7 Video Capture and Analysis

All the videos were captured and recorded with iPhone 4S (iPhone 4S from http://store.apple.com/us/buy-iphone/) and uploaded to computer (Toshiba Satellite R845). The iPhone is able to capture video at 30 frames per second with a resolution of 1920 x 1080 pixels by using the Camera application; this application had standard settings for white balance which was maintained throughout all of the experiments. For capturing the video of experiments, consistant overhead flourescent lighting was used. The iPhone was mounted on a tripod as close as possible to the Dynamic Magnetic Trap Bioreactor while staying in focus. The iPhone has an autofocus function and has a limit to how close an object is to the lens before it goes out of focus. To increase the contrast of the beads in solution, a sheet of white printer paper was folded and placed behind the cuvette. This arrangement was maintained throuhout all experiments in order to standardize all experimental conditions such as lighting and white levels.

The video files are uploaded to my computer and then imported into ImageJ (http://rsbweb.nih.gov/ij/download.html) using a QuickTime Capture plugin (http://rsbweb.nih.gov/ij/plugins/index.html). This plugin converts the imported video into a stack of images. The numerical data was obtained from the video using ImageJ. All of the numerical data was analyzed and plotted using Excel.

When running experiments at this scale, it become challenging to quantify what is being observed in the micro cuvettes. Using the video and ImageJ software, a method was devised to analyze the motion of the micro magnetic beads. The area between the electromagnets, where the steady-state mixing occurs, is selected and a Histogram analysis is performed across the entire stack of images. This area selected had dimensions of 70 pixels x170 pixels, which is a total of 11,900 pixels analyzed. This area was between the electromagnets that excluded the edges of the micro cuvette. These dimension were help constant over all of the experiments. The Histogram analyzes the level of grey in each pixel in the selected area and output the total pixel count, and the mean, standard deviation and the max/min of the greyscale values for the selected area. Histogram value has a scale from 0 to 256, where a value of 0 and 256 would respectively indcate black and white. For this project we will focus on the mean greyscale. Depending on how many micro magnetic beads were trapped once steady state mixing occurs, ImageJ will assign a mean greyscale number. For a stack of images from the imported video, ImageJ will average this value over the entire stack. The lower the mean greyscale number the darker the area where mixing of micro magnetic beads occurred. This correlates directly to magnitude of the magnetic flux density as higher flux densities would be able to trap more micro magnetic beads than lower magnetic flux densities.

V. Results

V.1 Time Until Steady State Mixing is Achieved

In these experiments, the time needed to achieve a steady state condition was recorded was recorded for each set of experiments. The steady state condition was defined as the state when no more micro magnetic beads are trapped in between the electromagnets. Figure 4 displays the steady state curves for all of the different steady-state experiments with the solution having a mass concentration of $\rho = 10$ g/L. The time until the steady state condition was recorded for each voltage and each frequency investigeted. Table 2 shows the numerical data from these experiments.

Table 2. The time in seconds elapsed until steady-state mixing for different frequencies

	Voltage Across Electromagnet [V]			
Frequency [Hz]	6	8	10	12
0.17	28.88	50.3	45.47	38.27
0.33	44.58	39.84	29.92	23.53
2	73.67	53.44	33.33	25.50
Average	49.04	47.86	36.24	29.10
Standard Deviation	18.56	5.81	6.67	6.53

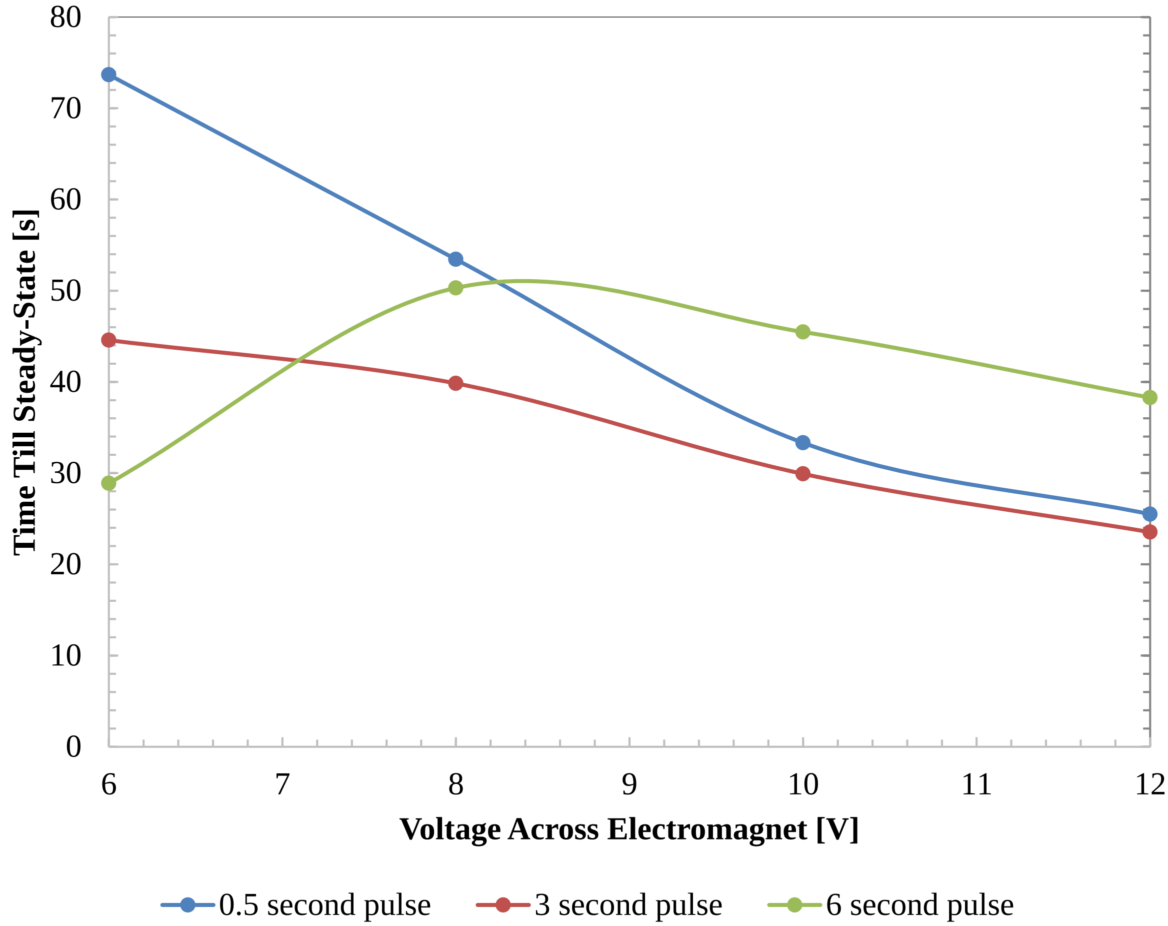

Figure 4. With the electromagnet working at frequencies of 2, 0.33, and 0.17 Hz, the time until steady-state mixing was recorded for the electromagnet operating at 2, 4, 6, 8, 10 and 12 Volts. The higher the voltage the faster the steady-state mixing is achieved.

V.2 Examining the Motion of Mico Magnetic Beads

At the electromagnets pulsing at f = 2 Hz, the change in magnetic flux density for the electromagnets operating from 2-12 volts. The experiment is repeated for each of the frequencies listed in table 1. The results can be seen in Figure 5.

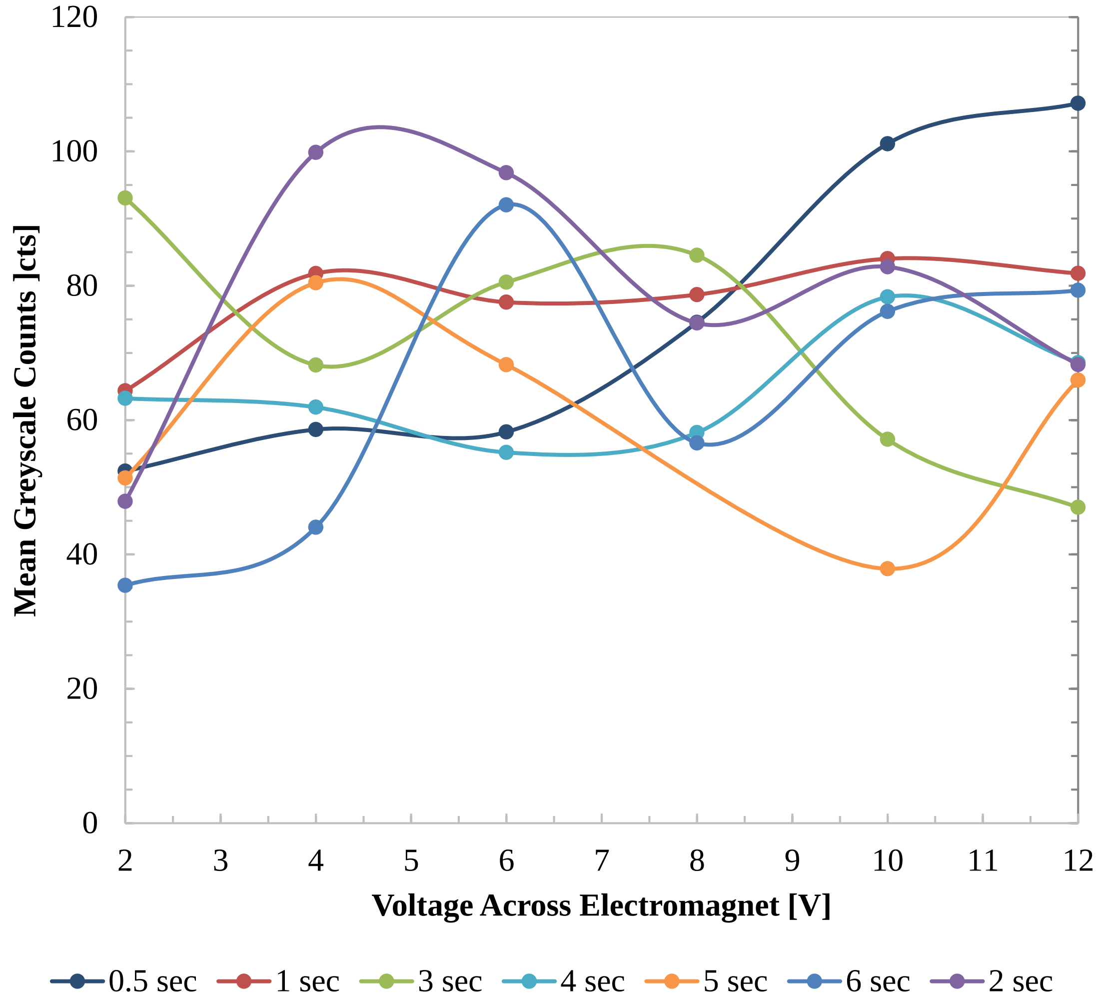

Figure 5. For a given pulse rate, video footage of steady-state mixing was captured for the electromagnet operating at 2, 4, 6, 8, 10, & 12 Volts; ImageJ was used to determine the mean Greyscale value of the area where the steady-state mixing occurred. For a given combination of pulse rate of the electromagnet and the voltage it is operating at, it will have a certain magnetic flux density.

The data for these experiments are listed in Table 3 below.

Table 3. Mean Greyscale Values of Steady-State Mixing for different voltages and frequncies at mass concentration of 10g/L

	Frequency [Hz]						
Voltage Across Electromagnet [V]	**2**	**1**	**0.5**	**0.33**	**0.25**	**0.20**	**0.17**
12	107.15	81.83	68.26	47.02	68.55	65.95	79.31
10	101.13	84.02	82.82	57.12	78.32	37.87	76.17
8	74.55	78.67	74.45	84.53	58.13	--	56.59
6	58.22	77.54	96.81	80.53	55.18	68.24	92.03
4	58.60	81.81	99.84	68.19	61.91	80.43	44.03
2	52.39	64.34	47.88	93.05	63.23	51.38	35.38

Table 4. Mean Greyscale Values of Steady-State Mixing at mass concentrations 5, 10 & 20g/L with the electromagnet operating at different voltages and a f = 1Hz

	Voltage Across Electromagnet [V]					
Mass Concentration	12	10	8	6	4	2
10 g/L	81.83	84.02	78.67	77.54	81.81	64.34
5 g/L	85.48	--	90.79	85.39	64.14	71.68
20 g/L	41.40	29.76	64.37	52.45	32.88	30.23

For a frequency of 1.0 Hz the change in motion of the micro magnetic beads was observed for the solution at mass concentrations $\rho = 5$ g/L and $\rho = 20$ g/L with the electromagnet operating with voltages 2-12 and compared to the values of the concentration of 10g/L. Table 4 list the values of the greyscale. The results can be seen in Figure 6.

All of these experiments were performed one time(N=1).

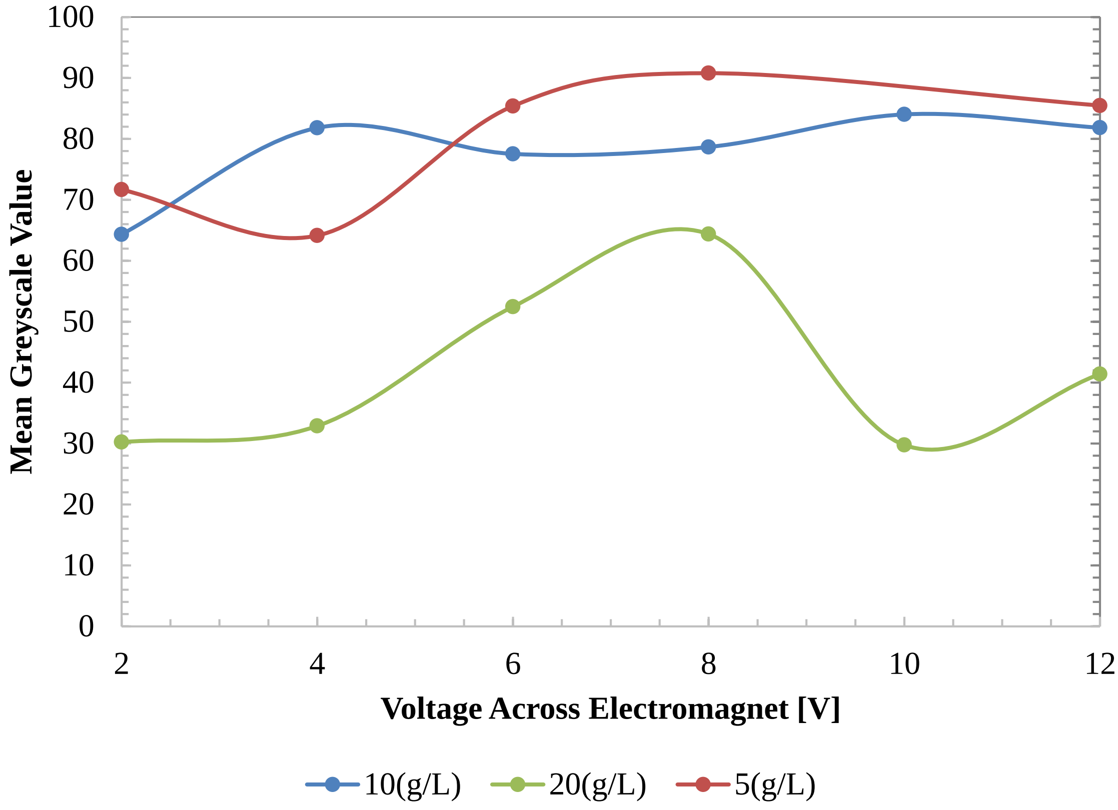

Figure 6. With the electromagnet working at a pulse-rate of 1 second, ImageJ was used to determine the mean Greyscale for steady-state mixing at mass concentrations 5, 10 and 20 g/L. The higher the concentration of micro magnetic beads, the more micro magnetic beads become trapped in steady-state mixing.

VI Discussion

VI.1 Steady State

As magnetic field strength increases, increasing voltage on electromagnet, the amount of time until steady-state mixing is attained decreases. This holds true looking at the average time until steady-state mixing for different operating frequencies. The variation of times are consistent amongst the different operating frequency for each voltage except at 6 volts. Steady-state mixing appears to occur much faster at this voltage when the frequency of 6 second pulse rate. One possible explanation could be that by having one electromagnetic maintain its magnetic field for longer allows for micro magnetic beads to be trapped into steady-state mixing much faster despite running at a low voltage. Further investigation is required since this is not consistent at higher voltages. Operating the electromagnets at higher voltages and alternating magnetic fields every 3 seconds allows for steady-state mixing to be achieved the fastest.

VI.2 Motion of Micro Magnetic Beads

At pulse frequency of 2Hz at 12V Figure 7 shows there is a fully developed magnet field outline and almost all of the magnetic beads are pulled away from left side of the cuvette.

Figure 7. The outline of magnetic beads when the voltage across the electromagnet is 12V and operating at 2Hz.

In Figure 8 the motion has changed as the electromagnet is unable to pull all of the micro magnetic beads from the other side of the cuvette.

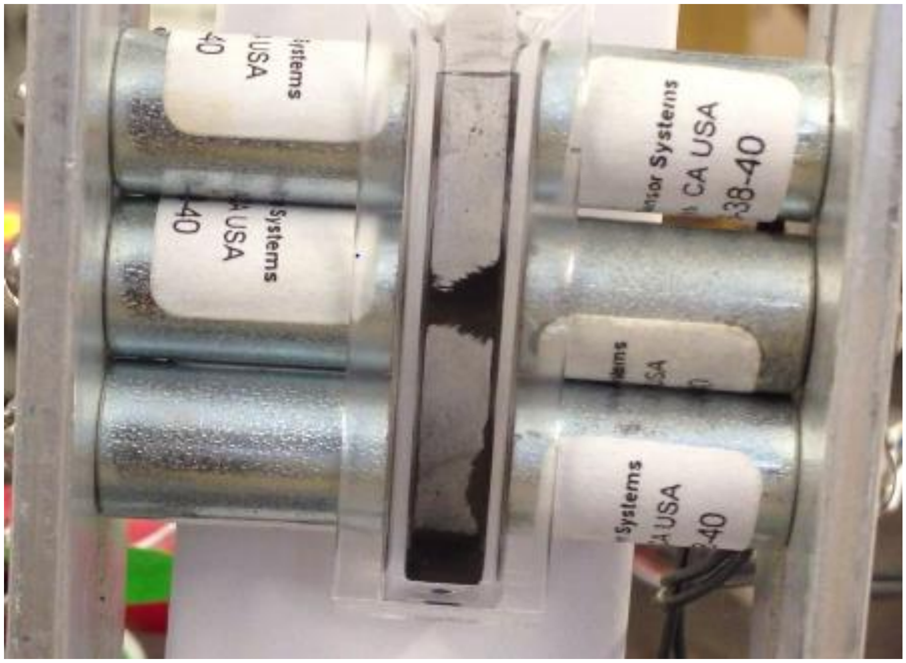

Figure 8. The outline of magnetic beads when the voltage across the electromagnet is 10V and operating at 2Hz.

In Figure 9, when the electromagnet is operating at 4 V, it just seems like some of the beads just stick to the side of the cuvettes and little to no magnetic beads are transferred side to side of the cuvette.

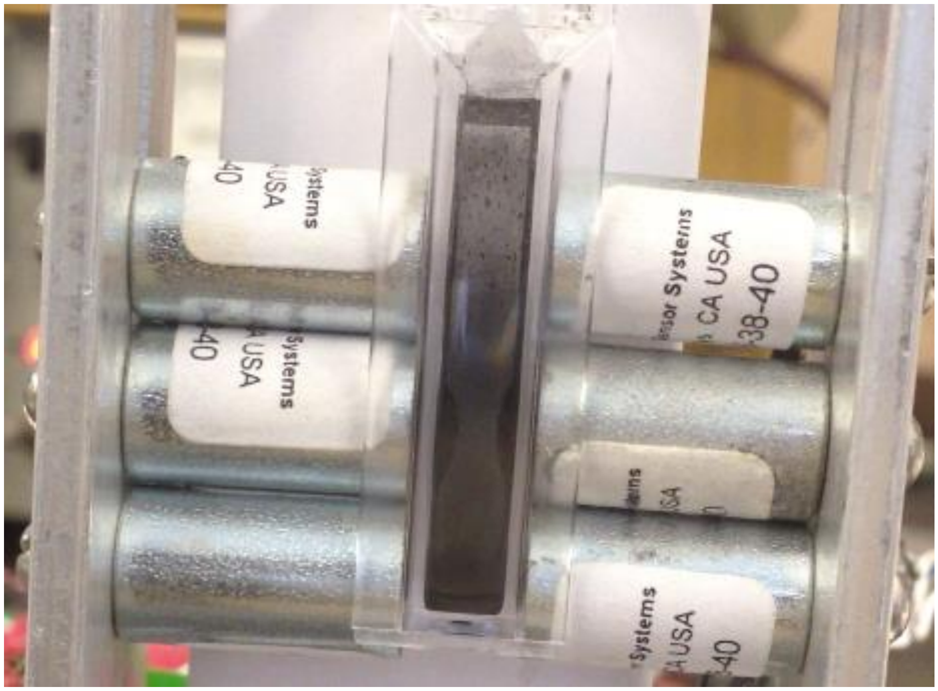

Figure 9. The outline of magnetic beads when the voltage across the electromagnet is 4V and operating at 2Hz.

For this voltage, we see that as there is a decrease in magnetic flux density as magnetic beads stick on the side of the cuvette. The greyscale value of 58.60 is much less than the value at 12V which is 107.15. Lower mean greyscale values indicate that there is much more black in the area that is analyzed. There are similar but reduced residues of magnetic beads at the side of cuvette near where the electromagnets are when operating at 2 and 4 volts. There is no visible transfer of the beads as the electromagnets pulse at these low voltages.

For the electromagnets operating at 1Hz, the micro magnetic beads behaved in a very similar as when the electromagnets are operating at 2 Hz. The greyscale values does not seem to portray this quantitatively as the do decrease and then increasing again. This is due to the fact that the micro magnetic beads are sticking to the side of the cuvettes as the mixing occurs to achieve steady state.

For when the electromagnets are pulsing at a frequency 0.33Hz, it behaves in a very similar way as the previous two pulse rates. However, it seems to not have trapped as many micro magnetic beads in steady-state mixing. Since one electromagnet produces a magnetic field on a side of the cuvette that micro magnetic beads are able to slip by the "trap" of the magnetic field along the edges of the cuvette that's not adjacent to the electromagnet. At weaker operating voltages, the magnetic flux density is much smaller in all dimensions allowing the magnet beads to simply fall toward the bottom.

There is a common trend for the steady-state mixing for when the electromagnets are pulsing at frequncies of 0.25, 0.20, and 0.17 Hz. When the electromagnets are running at 12 volts, significant amount micro magnetic beads are trapped in steady-state mixing. However when operating at voltages 10 through 2, there is a gradual decline in the amount of micro magnetic beads that are moving across the cuvette. Across these frequencies, when the electromagnets are running at 8, 6 and 4 volts a mound of micro magnetic beads that seem stuck to the side of the cuvette; and the amount of micro magnetic beads that travels back and forth decreases as the voltage lowers. At the lower frequencies of 0.20 and 0.17Hz, there appears to be little to no movement of beads when the electromagnets are running at 6, 4, and 2 volts. The greyscale values for these mixings are relatively similar in value confirming that this effect. The abnormal values can occur as a result of the micro magnetic beads become stuck to the cuvette during the process of attaining steady state mixing.

VI.3 Different Concentrations

The baseline mass concentration of $\rho = 10$ g/L was used in the previous experiment. When doubling the mass concentration to $\rho = 20$ g/L, the steady-state mixing changes. The steady-state greyscale values at this mass concentration are lower. Lower greyscale values correlate with much more black in the area the steady-state mixing occurs. This correlates directly with greater number of micro magnetic beads getting trapped between the electromagnets. This occurs because a higher number of micro magnetic beads are able to be trapped at this concentration. As observed in previaous experiments, the beads can become physically held in this region since similar values of greyscale are observed at both high and low voltages.

The greyscale values of steady-state mixing at mass concentration $\rho = 5$ g/L are higher than the baseline mass concentration. Higher greyscale values correlates with lower amounts micro magnetic beads being trapped. There are much less micro magnetic beads that can be trapped into steady-state mixing thus these values are expected. Since there are less micro magnetic beads, they travel across the cuvette.

VII Conclusion

Magnetic flux density, frequency, and mass concentration contribute greatly when controlling micro magnetic beads with attached enzymes and catalyst in solution with electromagnets. By controlling these micro magnetic beads in solution, this system can be utilized as a batch bioreactor with mixing controlled by the electromagnets. By examining how steady-state mixing is achieved, insights on how to control the micro magnetic beads were developed. The combination of high magnetic flux density, obtained with operating the electromagnets at higher voltage, and the right frequency allows for steady-state mixing to be quickly reached. The results from examining changing magnetic flux densities shows that higher densities creates better agitation levels. By adjusting the frequency, the rate at which mixing occurs can be controlled. Different mass concentrations of the micro magnetic beads change how quickly steady-state mixing is reached and the amount of agitation the beads undergo.

The results of this project illustrate how one can use electromagnets to control magnetic beads embedded with enzymes in a variety of ways. This can be used to design a magnetically controlled bioreactor with the micro magnetic beads embedded with enzymes and catalyst. These conclusions are a result from only one sample for each data point; therse experiments needs to be replicated more in order to make meaniful conclusions. Further research needs to be done to examine the effects of using stronger electromagnets and being able to control the magnet beads in a larger volume of solution. By examining these variable, insights on how to scale a bioreactor using these embedded micro magnetic beads.

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Appendix A. Code to control Electromagnets in the Dynamic Magnetic Trap Bioreactor

```
void setup() {
//pinMode(2, OUTPUT);
pinMode(3, OUTPUT);
//pinMode(4, OUTPUT);
//pinMode(5, OUTPUT);
pinMode(6, OUTPUT);
//pinMode(7, OUTPUT);

}

void loop() {
digitalWrite(3, LOW);
//digitalWrite(4, LOW);
digitalWrite(6, LOW);
//digitalWrite(7, LOW);
//digitalWrite(2, LOW);
//digitalWrite(5, LOW);
delay(000);
digitalWrite(3, HIGH);
//digitalWrite(4, HIGH);
//digitalWrite(2, HIGH);
delay(1000);
digitalWrite(3, LOW);
//digitalWrite(4, LOW);
//digitalWrite(2, LOW);
digitalWrite(6, HIGH);
//digitalWrite(7, HIGH);
//digitalWrite(5, HIGH);
delay(1000);
digitalWrite(6, LOW);
//digitalWrite(7, LOW);
//digitalWrite(5, LOW);

}
```

Appendix B. Specification Sheet for Tektronix 2012

Appendix: Warranted Specifications, Safety Certifications, and Electromagnetic Compatibility

Analog bandwidth	Oscilloscope	5 mV/div to 5 V/div with an ambient temperature of 0 °C to 40 °C (0 °F to 104 °F)	5 mV/div to 5 V/div with an ambient temperature of 0 °C to 50 °C (0 °F to 122 °F)	<5 mV/div
	DPO2024, MSO2024	DC to ≥200 MHz	DC to ≥160 MHz	20 MHz
	DPO2014, MSO2014, DPO2012, MSO2012	DC to ≥100 MHz		20 MHz
Input impedance, DC coupled	1 MΩ ±2% in parallel with 11.5 pF ±2 pF			
DC Balance	±(1 mV + 0.1 div)			
DC gain accuracy	±3%, 5 V/div through 10 mV/div ±4%, 5 mV/div and 2 mV/div			
Offset accuracy	± [0.01 × | offset – position | + DC Balance] *NOTE. Both the position and constant offset term must be converted to volts by multiplying by the appropriate volts/div term.*			
Long-term sample rate and delay time accuracy	±25 ppm over any >1 ms interval			
Digital Channel Threshold Accuracy, MSO2000 series only	± [100 mV + 3% of the threshold setting after calibration]			

Appendix C. Specification Sheet for Digitial Voltimeter Tek DMM 4020

DMM 4020
Users Manual

Electrical Specifications

Specifications are valid for 5-½ digit mode and after at least a half-hour warm-up.

DC Voltage Specifications

Maximum Input .... 1000 V on any range
Common Mode Rejection .... 120 dB at 50 or 60 Hz ±0.1% (1 kΩ unbalance)
Normal Mode Rejection .... 80 dB at Slow Rate
A/D Nonlinearity .... 15 ppm of range
Input Bias Current .... <30 pA at 25 °C
Settling Considerations .... Measurement settling times are affected by source impedance, cable dielectric characteristics, and input signal changes

Input Characteristics

Range	Full-Scale (5-1/2 Digits)	Resolution			Input Impedance
		Slow	Medium	Fast	
200 mV	199.999 mV	1 µV	10 µV	10 µV	>10 GΩ[1]
2 V	1.99999 V	10 µV	100 µV	100 µV	>10 GΩ[1]
20 V	19.9999 V	100 µV	1000 µV	1000 µV	10 MΩ±1 %
200 V	199.999 V	1 mV	10 mV	10 mV	10 MΩ±1 %
1000 V	1000.00 V	10 mV	100 mV	100 mV	10 MΩ±1 %

Notes:

[1] At some dual display measurements, the input impedance of 200 mV and 2 V ranges may be changed to 10 MΩ.

Accuracy

Range	Uncertainty [1]		Temperature Coefficient/°C Outside 18 – 28 °C
	90 days	1 year	
	23 °C ± 5 °C	23 °C ± 5°C	
200 mV	0.01 + 0.003	0.015 + 0.004	0.0015 + 0.0005
2 V	0.01 + 0.002	0.015 + 0.003	0.001 + 0.0005
20 V	0.01 + 0.003	0.015 + 0.004	0.0020 + 0.0005
200 V	0.01 + 0.002	0.015 + 0.003	0.0015 + 0.0005
1000 V	0.01 + 0.002	0.015 + 0.003	0.0015 + 0.0005

Notes:

[1] Uncertainty given as ± (% of reading + % of range)

AC Voltage Specifications

AC Voltage specifications are for ac sinewave signals >5 % of range. For inputs from 1 % to 5 % of range and <50 kHz, add an additional error of 0.1 % of range, and for 50 kHz to 100 kHz, add 0.13 % of range.

Maximum Input .... 750 V rms or 1000 V peak or 8 x 10^7 Volts-Hertz product

Measurement Method .... AC-coupled true-rms. Measures the ac component of input with up to 1000 V dc bias on any range.

AC Filter Bandwidth .... 20 Hz – 100 kHz

Common Mode Rejection .... 60 dB at 50 Hz or 60 Hz (1 kΩ unbalance)

Maximum Crest Factor .... 3:1 at Full Scale

Additional Crest Factor Errors (<100 Hz) .... Crest Factor 1-2, 0.05 % of full scale
Crest Factor 2-3, 0.2 % of full scale
Only applies for non-sinusoid signals

Input Characteristics

Range	Full-Scale (5-1/2 Digits)	Resolution			Input Impedance
		Slow	Medium	Fast	
200 mV	199.999 mV	1 µV	10 µV	10 µV	1 MΩ ±2 % shunted by <100 pf
2 V	1.99999 V	10 µV	100 µV	100 µV	
20 V	19.9999 V	100 µV	1000 µV	1000 µV	
200 V	199.999 V	1 mV	10 mV	10 mV	
750 V	750.00 V	10 mV	100 mV	100 mV	

Accuracy

Range	Frequency	Uncertainty [1]		Temperature Coefficient/°C Outside 18 – 28 °C
		90 days 23 °C ± 5 °C	1 year 23 °C ± 5 °C	
200 mV	20 Hz – 45 Hz	0.8 + 0.05	0.9 + 0.05	0.01 + 0.005
	45 Hz – 20 kHz	0.15 + 0.05	0.2 + 0.05	0.01 + 0.005
	20 kHz – 50 kHz	0.3 + 0.05	0.35 + 0.05	0.01 + 0.005
	50 kHz – 100 kHz	0.8 + 0.05	0.9 + 0.05	0.05 + 0.01
2 V	20 Hz – 45 Hz	0.8 + 0.05	0.9 + 0.05	0.01 + 0.005
	45 Hz – 20 kHz	0.15 + 0.05	0.2 + 0.05	0.01 + 0.005
	20 kHz – 50 kHz	0.3 + 0.05	0.35 + 0.05	0.01 + 0.005
	50 kHz – 100 kHz	0.8 + 0.05	0.9 + 0.05	0.05 + 0.01
20 V	20 Hz – 45 Hz	0.8 + 0.05	0.9 + 0.05	0.01 + 0.005
	45 Hz – 20 kHz	0.15 + 0.05	0.2 + 0.05	0.01 + 0.005
	20 kHz – 50 kHz	0.3 + 0.05	0.35 + 0.05	0.01 + 0.005
	50 kHz – 100 kHz	0.8 + 0.05	0.9 + 0.05	0.05 + 0.01
200 V	20 Hz – 45 Hz	0.8 + 0.05	0.9 + 0.05	0.01 + 0.005
	45 Hz – 20 kHz	0.15 + 0.05	0.2 + 0.05	0.01 + 0.005
	20 kHz – 50 kHz	0.3 + 0.05	0.35 + 0.05	0.01 + 0.005
	50 kHz – 100 kHz	0.8 + 0.05	0.9 + 0.05	0.05 + 0.01
750 V	20 Hz – 45 Hz	0.8 + 0.05	0.9 + 0.05	0.01 + 0.005
	45 Hz – 20 kHz	0.15 + 0.05	0.2 + 0.05	0.01 + 0.005
	20 kHz – 50 kHz	0.3 + 0.05	0.35 + 0.05	0.01 + 0.005
	50 kHz – 100 kHz	0.8 + 0.05	0.9 + 0.05	0.05 + 0.01

Notes:

[1] Uncertainty given as ± (% of reading + % of range)

Resistance

Specifications are for 4-wire resistance function, or 2-wire resistance with REL. If REL is not used, add 0.2 Ω for 2-wire resistance plus lead resistance.

Measurement Method .... Current source referenced to LO input

Max Lead Resistance (4-wire ohms) .... 10 % of range per lead for 200 Ω, 2 kΩ ranges. 1 kΩ per lead on all other ranges.

Input Protection .... 1000 V on all ranges

Input Characteristics

Range	Full-Scale (5-1/2 Digits)	Resolution			Current Source
		Slow	Medium	Fast	
200 Ω	199.999 Ω	0.001 Ω	0.01 Ω	0.01 Ω	0.8 mA
2 kΩ	1.99999 kΩ	0.01 Ω	0.1 Ω	0.1 Ω	0.8 mA
20 kΩ	19.9999 kΩ	0.1 Ω	1 Ω	1 Ω	0.08 mA
200 kΩ	199.999 kΩ	1 Ω	10 Ω	10 Ω	0.008 mA
2 MΩ	1.99999 MΩ	10 Ω	100 Ω	100 Ω	0.9 µA
20 MΩ	19.9999 MΩ	100 Ω	1 kΩ	1 kΩ	0.16 µA
100 MΩ	100.000 MΩ	1 kΩ	10 kΩ	10 kΩ	0.16 µA ‖ 10 MΩ

Accuracy

Range	Uncertainty [1]		Temperature Coefficient/°C Outside 18 – 28 °C
	90 days	1 year	
	23 °C ± 5 °C	23 °C ± 5 °C	
200 Ω	0.02 + 0.004	0.03 + 0.004	0.003 + 0.0006
2 kΩ	0.015 + 0.002	0.02 + 0.003	0.003 + 0.0005
20 kΩ	0.015 + 0.002	0.02 + 0.003	0.003 + 0.0005
200 kΩ	0.015 + 0.002	0.02 + 0.003	0.003 + 0.0005
2 MΩ	0.03 + 0.003	0.04 + 0.004	0.004 + 0.0005
20 MΩ	0.2 + 0.003	0.25 + 0.003	0.01 + 0.0005
100 MΩ	1.5 + 0.004	1.75 + 0.004	0.2 + 0.0005

Notes:

[1] Uncertainty given as ± (% of reading + % of range)

DC Current

Input Protection .... Tool accessible 11 A / 1000 V and 440 mA / 1000 V fuses.

Shunt Resistance.. 0.01 Ω for 2 A and 10 A ranges
1 Ω for 20 mA and 200 mA
Burden voltage < 5 mV for 200 µA and 2 mA range.

Input Characteristics

Range	Full-Scale (5-1/2 Digits)	Resolution			Burden Voltage
		Slow	Medium	Fast	
200 µA	199.999 µA	0.001 µA	0.01 µA	0.01 µA	<5 mV
2 mA	1999.99 µA	0.01 µA	0.1 µA	0.1 µA	<5 mV
20 mA	19.9999 mA	0.1 µA	1 µA	1 µA	<0.05 V
200 mA	199.999 mA	1 µA	10 µA	10 µA	<0.5 V
2 A	1.99999 A	10 µA	100 µA	100 µA	<0.1 V
10 A	10.0000 A	100 µA	1 mA	1 mA	<0.5 V

Accuracy

Range	Uncertainty [1] 90 days 23 °C ± 5 °C	Uncertainty [1] 1 year 23 °C ± 5 °C	Temperature Coefficient/°C Outside 18 – 28 °C
200 µA	0.02 + 0.005	0.03 + 0.005	0.003 + 0.001
2 mA	0.015 + 0.005	0.02 + 0.005	0.002 + 0.001
20 mA	0.03 + 0.02	0.04 + 0.02	0.005 + 0.001
200 mA	0.02 + 0.005	0.03 + 0.008	0.005 + 0.001
2 A	0.05 + 0.02	0.08 + 0.02	0.008 + 0.001
10 A	0.18 + 0.01	0.2 + 0.01	0.008 + 0.001

Notes:

[1] Uncertainty given as ± (% of reading + % of range)

AC Current

The following ac current specifications are for sinusoidal signals with amplitudes greater than 5 % of range. For inputs from 1 % to 5 % of range, add an additional error of 0.1 % of range.

Input Protection .... Tool accessible 11 A / 1000 V and 440 mA / 1000 V fuses

Measurement Method .... AC-coupled True RMS

Shunt Resistance .... 0.01 Ω for 2 A and 10 A ranges
1 Ω for 20 mA and 200 mA

AC Filter Bandwidth .... 20 Hz – 100 kHz

Maximum Crest Factor .... 3:1 at Full Scale

Additional Crest Factor Errors (<100 Hz) .... Crest Factor 1-2, 0.05 % of full scale
Crest Factor 2-3, 0.2 % of full scale
Only applies to non-sinusoid signals

Input Characteristics

Range	Full-Scale (5-1/2 Digits)	Resolution Slow	Resolution Medium	Resolution Fast	Burden Voltage
20 mA	19.9999 mA	0.1 µA	1 µA	1 µA	<0.05 V
200 mA	199.999 mA	1 µA	10 µA	10 µA	<0.5 V
2 A	1.99999 A	10 µA	100 µA	100 µA	<0.1 V
10 A	10.0000 A	100 µA	1 mA	1 mA	<0.5 V

Accuracy

Range	Frequency	Uncertainty [1] 90 days 23 °C ± 5 °C	Uncertainty [1] 1 year 23 °C ± 5 °C	Temperature Coefficient/°C Outside 18 – 28 °C
20 mA	20 Hz – 45 Hz	1 + 0.05	1.25 + 0.08	0.015 + 0.005
	45 Hz - 2 kHz	0.25 + 0.05	0.3 + 0.08	0.015 + 0.005
200 mA	20 Hz – 45 Hz	0.8 + 0.05	1 + 0. 08	0.015 + 0.005
	45 Hz - 2 kHz	0.25 + 0.05	0.3 + 0.08	0.015 + 0.005
2 A	20 Hz – 45 Hz	1 + 0.05	1.25 + 0.08	0.015 + 0.005
	45 Hz - 2 kHz	0.25 + 0.05	0.3 + 0.08	0.015 + 0.005
10 A	20 Hz – 45 Hz	1 + 0.1	1.25 + 0.12	0.015 + 0.005
	45 Hz - 2 kHz	0.35 + 0.1	0.5 + 0.12	0.015 + 0.005

Notes:

[1] Uncertainty given as ± (% of reading + % of range)

Frequency

Gate Time .... 131 ms

Measurement Method .... AC-coupled input using the ac voltage measurement function.

Settling Considerations .... When measuring frequency after a dc offset voltage change, errors may occur. For the most accurate measurement, wait up to 1 second to allow input blocking RC time constant to settle.

Measurement Considerations .... To minimize measurement errors, shield inputs from external noise when measuring low voltage, low frequency signals.

Accuracy

Range	Frequency	Uncertainty		Temperature Coefficient/°C Outside 18 – 28 °C
		90 days	1 year	
		23 °C ± 5 °C	23 °C ± 5 °C	
100 mV to 750 V[1,2]	20 Hz – 2 kHz	0.01 + 0.002	0.01 + 0.003	0.002 + 0.001
	2 kHz – 20 kHz	0.01 + 0.002	0.01 + 0.003	0.002 + 0.001
	20 kHz – 200 kHz	0.01 + 0.002	0.01 + 0.003	0.002 + 0.001
	200 kHz – 1 MHz	0.01 + 0.004	0.01 + 0.006	0.002 + 0.002

Notes:

[1] Input > 100 mV

[2] Limited to 8* 10^7 V Hz

Continuity

Continuity Threshold .... 20 Ω

Test Currents .... 1 mA

Response Time .... 100 samples/sec with audible tone

Rate .... Fast

Maximum Reading .... 199.99 Ω

Resolution .... 0.01 Ω

Diode Test

Response Time .... 100 samples/sec with audible tone

Rate .... Fast

Maximum Reading .... 1.9999 V

Resolution .... 0.1 mV